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TEednloin ormdnoE · hamemnent of prolifemdon and cytotoxidtyaggdnst lymphokine-activated killer cell human bladder cancer cells in

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KEY WORDS' o ; vitamin A; lymphokine-activa d kiler ωlls; n division; interlel n-2; immunologic cytotoxicity; cultured tnmor cells; bladder n 'Plωms

.AIM::To sωdy e eflect of tre. oin (Tre) or re .o1 (Ret) on e proliferation of lymphok:ine-activa ller (LAK) cells in pa with transitional oell mωrof bIBddermd ekcyω11ysisωbladde mmOJT ωHs. ME1 :ODS: LAK 0011 proliferation was assayed in e presence of ei er Tre or'Ret by ceU m g. HUlnan 3nsitional bladderωncer cell lines B '-87 or bladder tnruor cells (BTC) from patients wi bladderωncer were used as rget cells and cytotoxici of LAK cells was detelluined by L Dime-yl iazol-2-yl) 5-diphenyltetr olium bromide (Mπ) assay. RI ULTS: The proliferation of LAK ce1 1 sduood by interleu n-2 (1L-2) was stimu1!a by Tre or Ret (10-100 nmol. L-1) - le cyωωlxicity of LAK ceHs against BIU-87 FJ cells or BTC was e mωd by pretrea1ment of LAK cells wi Tre or Ret 10 - 1 nmol•L1. CONCLUSION: Tre or Ret enbances e oliferation and c otoxicity of LAK cells from patil .ts wi bladder cancer. Re oi e potential in adoptive immunotherapy of bladder ωncer.

Inrlividuals wi low serum re loids levels or low dietary intake of retinoids have an inoreased risk of bladder cancer and retinoids prevent e emergence of bladder cancer m carcinogen treated mmme 1- Retinoids inhibit proliferation induce differentiation or apopoωsis and regulate gene expression in various ωncer cells[2-4]ω. Yet . ere e few reports about the effec of retinoil .on antiωmor immunity in human. The immunologic aberrations induced by tumors in mice with implan d melanoma weredmhMedh tretinoin indicating t noidsmaykof15 in cancer immunotherapy([5-8]).

ηle results by using lympbo ne-activated b (LAK) cells in clinical practice were IDsatisfactory(9) t was i!rnpo all tω denti cb agent was endow with an imm.unostimulatory effect. 1s study was to investigate the ffect of Tre or retinol (Ret) in e generation of lAK. cells in patients wi 1 bladder ωnc and e cytotoxicity of lAK. cells against bladder cancer cells.

MATERIALS AND METIlODS

Reagents Tre and Ret were made by Fluka (Switzedand). RPMI 164 medlml was obtained from. Gibco. 3-5-Din lethyl iazol-2-yl)5- diphenylte rolium bromi (Mπ) and *L*-glutamine were obtained from Si a. Recombinant interleuk:in-2 (-2) was made byαangchID Institute of Biologic Pr lC (Miniy of PublicH C).

Culti."èltion of LAK cells Peripheral b mononuclear cells (PBMC) obtained oII) 19 patierr wi pa ologil ly diagnosed transitional cell carcinoma of bladder were isola1 by FicoN-paque (Shanghai 3 Chemical .R! gents Ltd) density-.ruent cenlriiugation. Irt! faGe cells weEe pirated washed 3 llimes wi 1 solu on. The PBMC (1x 1(f cells -L-1) were suspended in complete medium (CM) consisting RPMI 1640benzylpenicillin 1 kU'L -lLstreptomycin 100 kU -L-1 gentarnicin 50 kU'L-1 *L*-glutamine 2 mmol'L s ium p uvate 1 rnmol'L-1 and 15 % heat-inactiva dfe calf sennn < S).The cells were allowed to se e'in 25 cnr tissue culture fl k8 at 37 in 5 %α for 2 h. nonadherent PBMC (2 x 1 cells'L-1) were rther cultivaJd in CM supplemented with IL-2 1MU'L-1 for 96 h.

LAKceft prøl eration say η1.e nωωonadheorent PBMC (15 x 1θ. c ed ed Jrrs/well îd 3ωμLαα + IL-2 we replω d

Wel1plat h .e presenωcωe ofei e' 'fre -100 ll'L Ret (ω0-10 nol·1.-). Eaah nωntration had'4 wells. 1bC

LWt mMWh nune from the' State Ed øn u>mmission of αina and 1 Science Foundation of Gansu Provinω(ZG- 12).

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dlzbrs rially counted Ul a hemocytometer. The lests were W ωlcu1ated inωch a ne IInw of ertment.

4 :e.dqawBidd Mor cells ( ) from ı .mnsiuo ωU cinoma of bladdor were prepared r HIEnm bladder mmdoMcellcm inormd

wBWT : :2:rct:zz

sftffkd(0 mmol-U)b h and washed with j v·.lweze added tomrFt cells in dketor/wget

WW :lin250 e c of LAK Z;;;ed by MIT ω ωrepea independent JdnM I4 wells each). le abs.o ance (A) at 570 mn in TZL rnùned by rm oreader (-v'IKMWP[1-(AofmFt us LAK-A ;Ed of 1% Slat S s IAK 0011 prO era1 l i m ear 00 wi. ANOVA. All LAK cell cyωω αty ag W:ils tUJ Of ceßs was analyzed by I-I 8l..

RESULTS

LAK cell grow UK ceils wereuea Tre (0-10μmol·L^{-1}) or Ret (10μnol.L^{-1}) %h.'IEe pmHtendon ofIAkdshducdby -2 was stimulated by Tre or Ret at 10 1 nmol. L^{-1} m48ω96h (Fig 1).

Und - beyond concéntration r ge ere e 00 stimulatilω(Tab ▮ $P < O.Đ$) .

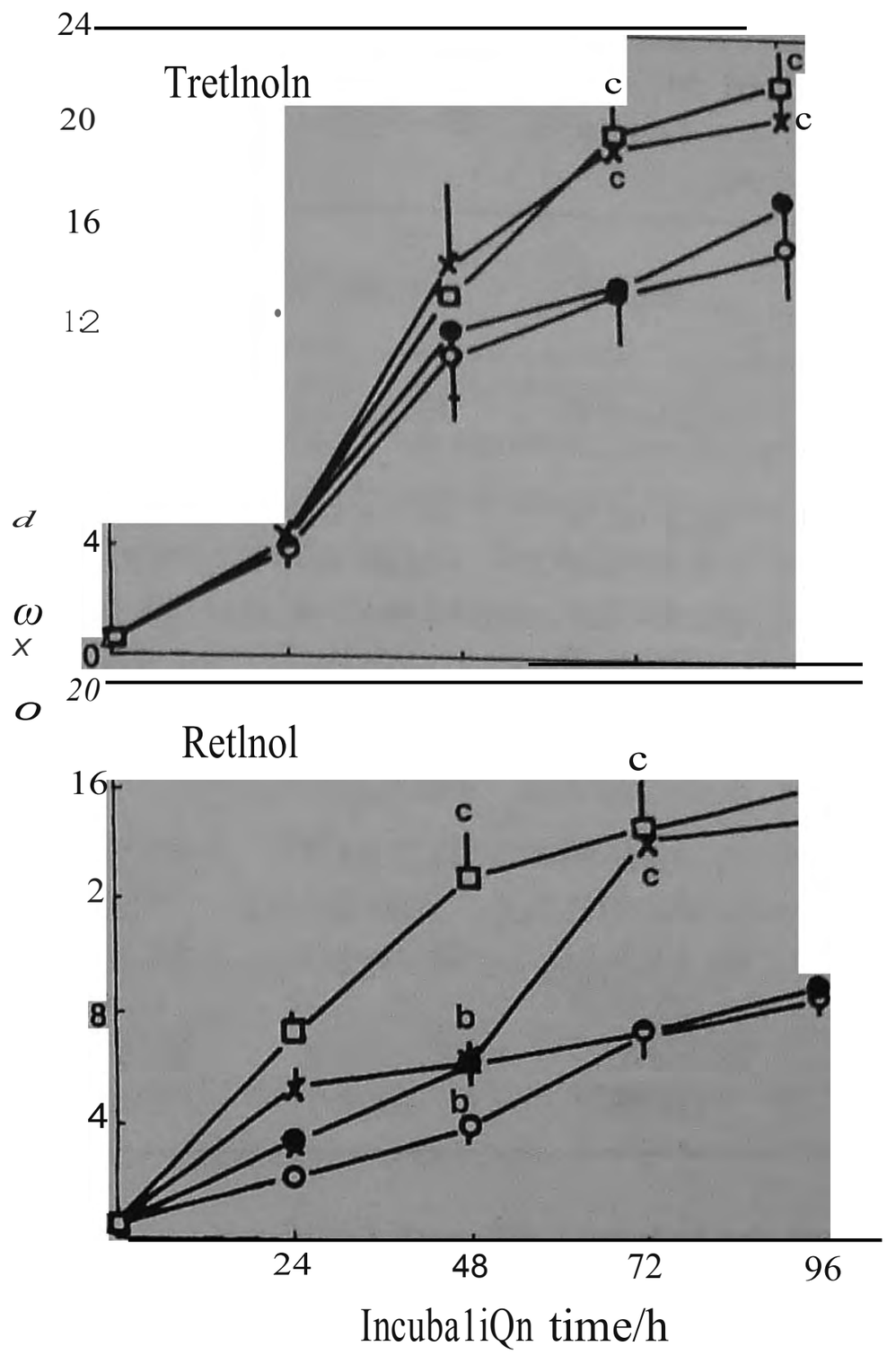
Tab 1 *LAK cell mmiJ.ber after eatmmt wi 1L.2 iin combinatü -mwi Tre or Ret. n 4 wellS and repeateà*

4 S.

bp<0.05 cp<0.01 vs control.

Tre or Ret/ nmol.L^{-1}	UIlllIli{10^{7} x cells.L^{-1}) Tre oin	Retinol
0	156	89 4
1	175 20	93 4
10	2 1ō^{b}	154 6^{c}
1	22213^{c}	163:1:9^{c}
lαm	154 16	73 3
10	121:t:].i	72 3

cell Cytotoxicity of LAK cells against twnor nsitional cell S The LAK cells from tien varying conc of bla er were pre d Wi 1L-2 plus or Ret Tre or Ret significantly !e ations of Tn BIU-87 e deyωtoxicity of LAKωHs agaiast enhancement ω118 Qr Bl'C res c vely and the



f'ig 1. E t of tre. 10in ør retinol on LAK cell prnlif2 on duced by IL-2. The LAK cells were atedwi 1L-2 1 MU.L^{-1} aløne {O). n .1+Tre or ltet at t " 110 (alld 1 : 1unol.L^{1}' (J. n= 4 weDsVgroup and re d for 5 iDde ø experiments. bp <0.05. cp <0.01 vs JL.2 group.

BID-87 and ET (Tab 2) – le treatment. df EJ B 87 or Btc wi Tre or Ret 1 nmol·L^{-1} for 4 h did nQt affect the A of MTf

DISCUSSION

iBx essioß of e' ne reoo1tor α mRN:A can be deteoted m antigen-s nula: d' T lyrnphocytes and Tre stimulated activated 1 ' nph esωproliferation in a dose dependent manned7 .L2·induced UK cen actîγity was enhanced bytm m miω.(8) 11 for BTC is tgωt mp edwi

all ωe expe nents were fωned in animal lyrnph ytes 80 tlhe effects of Treor Ret on the proMmtion of Mm LAK prOÜD n of LAK cells induced by 1L-2 mm ents wi bladder was s ulæ d by the mn Incentrat 1s of TreωRδt. sho 111g t combination ofIL-2 witb Tre or Ret has synergis c

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bp< cp<0.01

αmtrol.

	BIU. . 7	EJ	BTC
Conωl	47.6 :t:3.8	48.3 3.5	38.0 4.4
Tretinoin			
1	52.2 :t:2.1 (110 %)8	53.1 4.8 (110 %)a	82.4 2.4 (216 %)C
10	56.4 3.5 (118 %)b	57.4 3.9 (119 %)b	89.4 2.9 <5 %)C
1	ω.0 2.9 (126 %)C	61.1 2.6 (126 %)C	95.2 3.7 (250 %)C
Retinol			
L	45.8 2:4 (96 %)a	47.2 1.3 (98 %)a	37.2 1.6 (98.8)a
10	59.8 4.1 (126 %)b	58.3 4.9 (121 %)b	57.2 :t: 3.7 (150 %)b
1	64.3 :t: 5.7 (135 %)C	62.7 4.8 (130 %)C	61.9 :t: 3.4 (163 %)C

action on the human t i\K cell proliferation.

0ur study demonstrated that cytotoxicity of LAK cells against BIU-81 EJ cells or B'fC from e patients was enhanced by Tre oT Ret. lese IEsults wem mpatible with .088 ɗ e eriments[ß) lfat Tre au lented 1AK 0011 activity in 00 cQncen atiom- and time-dq:ndent manners in mbination wi IL-2. This indicates at retinoids 'e potential in adoptive immuno erapy of bladder cancer.

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A A 1

23 24 3 3

(273 ;3

73 30)

A ; A;

; ; -2;

; ;

: A (Tre) A (Ret)

(LAK)

- :

M LAK

- : Tre Ret 10-1 nmol'L..1

(IL 2) LAK

- : Tre

Ret LAK

-

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